Introduction: Structural Properties for Determining Mechanisms of Toxic Action

by Steven P. Bradbury* and Robert L. Lipnick†

The U.S. Environmental Protection Agency (EPA), under a variety of Federal legislation, is charged with the responsibility of assessing the hazards of chemicals to human health and the environment. In some cases EPA incorporates predictive techniques in its decision-making processes. In the context of some statutes, predictive toxicological methods can be cost-effective components in an overall approach for prioritizing chemicals for in-depth toxicological investigation. Predictive approaches are also used where empirical toxicological data are either unavailable or not required under a specific statute. For example, under Section 5 of the Toxic Substances Control Act (TSCA), EPA's Office of Toxic Substances must review and assess the potential hazard of a new industrial chemical within 90 days, generally with little more available information than the compound's structure. Although submitters are required to provide EPA with any toxicological data available at the time of submission, they are not required to conduct additional testing unless the agency can demonstrate that a given chemical is "... likely to present an unreasonable risk to human health or to the environment."

Successful implementation of TSCA illustrates the need to establish reliable predictive techniques because laboratory resources are limited and the number of potential compounds for study is extremely large. In the field of environmental toxicology, and especially aquatic toxicology, quantitative structure-activity relationships (QSARs) have been developed as scientifically defensible and effective tools for predicting the toxicity of xenobiotics. Proper application of QSAR techniques, however, requires that models be generated for specific modes of toxic action and that methods be developed to systematically assign chemicals to the appropriate QSAR. Thus, the use of mechanism-based QSARs requires a fundamental understanding of both toxic mechanisms and the critical structural characteristics and

*United States Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MN 55804.

†United States Environmental Protection Agency, Office of Toxic Substances, Health and Environmental Review Division, 401 M. St., S.W., Washington, DC 20460.

Address reprint requests to S. Bradbury, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MN 55804.

properties of a chemical that govern its action by a specific mechanism.

As part of an on-going and cooperative effort in this area of research, a workshop entitled "Structural Properties for Determining Mechanisms of Toxic Action" was co-sponsored by the EPA through the Health and Environmental Review Division of the Office of Toxic Substances and the Environmental Research Laboratory—Duluth, of the Office of Research and Development. The goal of the workshop was not only to review current understanding of fundamental mechanisms, but also to develop an initial knowledge base on chemical features and properties from which toxic mechanisms could be predicted from structure. Areas addressed included general anesthesia or narcosis, oxidative phosphorylation uncoupling, electrophile and free-radical reactivity, and a variety of pesticide-based mechanisms. In addition to providing knowledge for an expert system designed to predict mechanism from chemical structure, the results of this workshop also serve as a unique compilation of information that should be of interest to toxicologists in general.

In the opening paper, Auer et al. provide insights into the procedures and criteria that the Office of Toxic Substances currently uses in its evaluation of chemicals under the Premanufacture Notice process of TSCA. The magnitude of the effort required for these hazard evaluations, under just one of the legislative mandates of the agency, underscores the usefulness of predictive toxicological approaches. In addition, the specific need for an understanding of the relationship between toxic mechanism and chemical structure in this regulatory setting is clearly presented.

After this introductory presentation, focus is shifted to a detailed examination of specific toxic mechanisms. In the next two papers the issue of general anesthesia or narcosis is addressed. This toxic mechanism is especially critical in an acute exposure scenario for aquatic organisms in that approximately 70% of monomeric industrial organic compounds (excluding pesticides and pharmaceuticals) are thought to act by narcosis. Franks and Leib provide a presentation of the mechanistic basis of general anesthesia and an assessment of the nature and potential sites of action. Veith and Broderius then

discuss the apparent dichotomy of simple nonelectrolytes into classes of nonpolar and polar narcotics and examine the mechanistic implications that follow. Rules are also provided for selecting the appropriate narcosis QSAR for a given chemical.

Veith and Broderius also suggest preliminary rules for distinguishing polar narcotics from industrial chemicals that are thought to act as uncouplers of oxidative phosphorylation. Terada then presents an overview of the protonophoric action of weakly acidic uncouplers and their structural characteristics. The critical roles of an acid dissociable group, a bulky hydrophobic moiety, and a strong electron-withdrawing group in an uncoupler are examined.

Hermens, Carlson, and Kadlubar et al. address a variety of issues regarding the identification and assessment of electrophiles, where acute and chronic toxic effects are a consequence of their binding to nucleophilic functional groups contained in biological macromolecules. Hermens provides a foundation for the systematic identification of chemical functional groups associated with electrophilic reactivity. He then relates this information to QSARs that have been developed to predict the acute toxicity of electrophiles to fish based upon a model reactivity parameter. Carlson addresses various approaches that can and have been applied to predict the reactivity of electrophiles, with an emphasis on DNA alkylation rates. The concept of using hard/soft acid-base theory as a theoretical basis for assessing carbon electrophiles is emphasized and the development of analytical techniques to assess reactivity and DNA site-specificity is outlined in terms of carcinogenicity and cytotoxicity. The electrophilic reactivity of many toxicants is, of course, the result of an activated metabolite(s). Kadlubar et al., using arylamines as model compounds, describe how the extent of metabolic N-oxidation (activation) in comparison to *ring* oxidation (detoxification) is correlated to the relative charge density on nitrogen versus *ring*-carbon atoms for a nitrenium/carbenium ion-enzyme intermediate. Further, they demonstrate that both half-wave oxidation potentials and the relative positive charge distribution at nitrogen versus carbon are useful electronic parameters for predicting the extent of metabolic activation to carcinogenic N-hydroxy arylamines.

Free-radical metabolites have also been implicated in a variety of toxic responses. Mason provides a review of the role of free-radicals in toxicology and describes how quantifiable redox properties could be useful predictors of reactivity.

In the final group of papers, several insecticide and herbicide classes are treated, where the mechanisms generally involve selective binding to specific receptor sites. Fukuto reviews the mechanism of acetylcholinesterase inhibition by organophosphorus and carbamate esters. The issue of electrophilicity is discussed again and inhibition of acetylcholinesterase is related to both chemical reactivity and steric requirements. Coats then examines the structural requirements of DDT-type, cyclodiene, and pyrethroid insecticides. In many instances examination of electronic and steric characteristics of active isomers has provided insights into the potential sites of action for these classes of neuroactive agents. Finally, Duke provides a systematic review of the large number of molecular mechanisms that have been exploited in the discovery and optimization of herbicidal activity. The ability to predict from chemical structure the molecular site of action of herbicides appears to be a great challenge.

In conclusion, the results of this workshop have provided important insights into certain aspects of chemical structure that are critically related to various mechanisms of toxic action. Obviously there is still much that needs to be explored. Equally important will be the application of computational chemistry techniques to accurately and rapidly calculate the required parameters needed to predict toxic mechanisms from structure.